

IN THE CLAIMS

Claims 62-64, 68, 69, 92, 100, 104, 120, 121, 128, and 129 have been canceled without prejudice or disclaimer. New claims 131-151 have been added. Claims 1, 52, 53, and 130 have been amended. Claims 1, 24, 32, 36, 52, 53, 60, 61, and 130-151 are pending in the present application. The following is the status of the claims of the above-captioned application, as amended.

1. (Currently Amended) A microbial trypsin variant having chymotrypsin-like activity, comprising one or more modifications selected from the group consisting of:

- (a) a substitution at one or more positions corresponding to positions 144, 193, 198, 201, 218, 223, 227, 228, 229, 230, and 231 of amino acids 25 to 248 of SEQ ID NO: 2;
- (b) a deletion at one or more positions corresponding to positions 192, 197, and 226 of amino acids 25 to 248 of SEQ ID NO: 2; and
- (c) an insertion between positions corresponding to positions 224 and 225 of amino acids 25 to 248 of SEQ ID NO: 2;

wherein the microbial trypsin is (a) a polypeptide ~~having~~ comprising an amino acid sequence which has at least 70% identity to amino acids 25 to 248 of SEQ ID NO: 2; or (b) a polypeptide encoded by a nucleotide sequence which hybridizes under at least low stringency conditions with nucleotides 202 to 801 of SEQ ID NO: 1 or its complementary strand, wherein low stringency conditions are defined as prehybridization and hybridization at 42°C in 5X SSPE, 0.3% SDS, 200 µg/ml sheared and denatured salmon sperm DNA, and 25% formamide followed by washing three times each for 15 minutes using 2X SSC, 0.2% SDS at 50°C, wherein the variant has chymotrypsin-like activity and has an amino acid sequence that has at least 70% identity to the amino acid sequence of the microbial trypsin.

2-23. (Cancelled)

24. (Original) The variant of claim 1, which comprises one or more substitutions selected from the group consisting of V144T, S193A, D198S, Q201M, A218I, N223S, R227S, P228T, N229S, Y230T, and S231P.

25-31. (Cancelled)

32. (Original) The variant of claim 1, which comprises one or more substitutions selected from the group consisting of V144T, S193A, D198S, Q201M, A218I, N223S, R227S, P228T, N229S, Y230T, and S231P; and one or more deletions selected from the group consisting of V192*, K197*, and A226*.

33-35. (Cancelled)

36. (Currently Amended) The variant of claim 1, which comprises one or more substitutions selected from the group consisting of V144T, S193A, D198S, Q201M, A218I, N223S, R227S, P228T, N229S, Y230T, and S231P; one or more deletions selected from the group consisting of V192*, K197*, and A226*; and ~~the an~~ insertion G224GT of amino acids 25 to 248 of SEQ ID NO: 2.

37-51. (Cancelled)

52. (Currently Amended) The variant of claim 1, wherein the total number of substitutions is at least 1 and at the most 11, ~~more preferably 10, even more preferably 9, even more preferably 8, even more preferably 7, even more preferably 6, even more preferably 5, even more preferably 4, even more preferably 3, even more preferably 2, and most preferably 1.~~

53. (Currently Amended) The variant of claim 1, wherein the total number of deletions is at least 1 and at the most 3, ~~more preferably 2, and most preferably 1.~~

54-59. (Cancelled).

60. (Original) The variant of claim 1, which is encoded by the nucleotide sequence contained in pEJG66.1XLGOLD which is contained in *E. coli*/NRRL B-30627.

61. (Currently Amended) The variant of claim 1, which is in ~~the a~~ form of a precursor comprising amino acids 1 to 24 of SEQ ID NO: 2 as ~~the a~~ prepro region, or a portion thereof, linked in translation reading frame with the amino terminus of the trypsin variant.

62-129. (Cancelled).

130. (Currently Amended) A detergent composition comprising a the variant of claim 1 and a surfactant.

131. (New) The variant of claim 1, which comprises the substitutions V144T + S193A + D198S + Q201M + A218I + N223S + R227S + P228T + N229S + Y230T + S231P.

132. (New) The variant of claim 1, which comprises the substitutions V144T + S193A + D198S + Q201M + A218I + N223S + R227S + P228T + N229S + Y230T + S231P, and the deletions V192* + K197* + A226*.

133. (New) The variant of claim 1, which comprises the substitutions V144T + S193A + D198S + Q201M + A218I + N223S + R227S + P228T + N229S + Y230T + S231P, and the deletions V192* + K197* + A226*, and the insertion G224GT of amino acids 25 to 248 of SEQ ID NO: 2.

134. (New) The variant of claim 1, wherein the microbial trypsin has an amino acid sequence which has at least 70% identity with amino acids 25 to 248 of SEQ ID NO: 2.

135. (New) The variant of claim 134, wherein the microbial trypsin has an amino acid sequence which has at least 75% identity with amino acids 25 to 248 of SEQ ID NO: 2.

136. (New) The variant of claim 135, wherein the microbial trypsin has an amino acid sequence which has at least 80% identity with amino acids 25 to 248 of SEQ ID NO: 2.

137. (New) The variant of claim 136, wherein the microbial trypsin has an amino acid sequence which has at least 85% identity with amino acids 25 to 248 of SEQ ID NO: 2.

138. (New) The variant of claim 137, wherein the microbial trypsin has an amino acid sequence which has at least 90% identity with amino acids 25 to 248 of SEQ ID NO: 2.

139. (New) The variant of claim 138, wherein the microbial trypsin has an amino acid

sequence which has at least 95% identity with amino acids 25 to 248 of SEQ ID NO: 2.

140. (New) The variant of claim 1, wherein the microbial trypsin has the amino acid sequence of amino acids 25 to 248 of SEQ ID NO: 2.

141. (New) The variant of claim 1, wherein the microbial trypsin is encoded by a nucleotide sequence which hybridizes under low stringency conditions with nucleotides 202 to 801 of SEQ ID NO: 1 or the nucleotide sequence of nucleotides 202 to 801 of SEQ ID NO: 1, wherein low stringency conditions are defined as prehybridization and hybridization at 42°C in 5X SSPE, 0.3% SDS, 200 µg/ml sheared and denatured salmon sperm DNA, and 25% formamide followed by washing three times each for 15 minutes using 2X SSC, 0.2% SDS at 50°C.

142. (New) The variant of claim 141, wherein the microbial trypsin is encoded by a nucleotide sequence which hybridizes under medium stringency conditions with nucleotides 202 to 801 of SEQ ID NO: 1 or the nucleotide sequence of nucleotides 202 to 801 of SEQ ID NO: 1, wherein medium stringency conditions are defined as prehybridization and hybridization at 42°C in 5X SSPE, 0.3% SDS, 200 µg/ml sheared and denatured salmon sperm DNA, and 35% formamide followed by washing three times each for 15 minutes using 2X SSC, 0.2% SDS at 55°C.

143. (New) The variant of claim 142, wherein the microbial trypsin is encoded by a nucleotide sequence which hybridizes under medium-high stringency conditions with nucleotides 202 to 801 of SEQ ID NO: 1 or the nucleotide sequence of nucleotides 202 to 801 of SEQ ID NO: 1, wherein medium-high stringency conditions are defined as prehybridization and hybridization at 42°C in 5X SSPE, 0.3% SDS, 200 µg/ml sheared and denatured salmon sperm DNA, and 35% formamide followed by washing three times each for 15 minutes using 2X SSC, 0.2% SDS at 60°C.

144. (New) The variant of claim 143, wherein the microbial trypsin is encoded by a nucleotide sequence which hybridizes under high stringency conditions with nucleotides 202 to 801 of SEQ ID NO: 1 or the nucleotide sequence of nucleotides 202 to 801 of SEQ ID NO: 1, wherein high stringency conditions are defined as prehybridization and hybridization at 42°C in 5X SSPE, 0.3% SDS, 200 µg/ml sheared and denatured salmon sperm DNA, and 50% formamide followed by washing three times each for 15 minutes using 2X SSC, 0.2% SDS at 65°C.

145. (New) The variant of claim 1, wherein the microbial trypsin is a wild-type microbial trypsin.
146. (New) The variant of claim 1, which has an amino acid sequence with at least 70% identity to the amino acid sequence of the microbial trypsin.
147. (New) The variant of claim 146, which has an amino acid sequence with at least 75% identity to the amino acid sequence of the microbial trypsin.
148. (New) The variant of claim 147, which has an amino acid sequence with at least 80% identity to the amino acid sequence of the microbial trypsin.
149. (New) The variant of claim 148, which has an amino acid sequence with at least 85% identity to the amino acid sequence of the microbial trypsin.
150. (New) The variant of claim 149, which has an amino acid sequence with at least 90% identity to the amino acid sequence of the microbial trypsin.
151. (New) The variant of claim 150, which has an amino acid sequence with at least 95% identity to the amino acid sequence of the microbial trypsin.